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Caribbean seagrasses as a food source for the emerald neritid *Smaragdia viridis*

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Abstract: Seagrass canopies harbor many different mollusc species, but information about the interaction of these seagrass residents with their host plants remains scarce. Most gastropods inhabiting seagrass meadows are believed to feed on epiphytes rather than directly on living seagrass tissues. In laboratory experiments, we demonstrate that the gastropod *Smaragdia viridis* (Linnaeus, 1758) feeds preferentially on three seagrass species that are common in the Caribbean and Bermuda, including *Thalassia testudinum, Halodule wrightii*, and *Syringodium filiforme*. The percentage of lysed over intact seagrass cells egested was significantly higher in gastropods fed either *T. testudinum* or *H. wrightii* (80.3 ± 4.7% and 84.6 ± 11.4%, mean ± *SD*) but not for *S. filiforme* (59.7 ± 15.9%). Diet versatility for both pioneer (*H. wrightii* and *S. filiforme*) and climax (*T. testudinum*) Caribbean seagrass species allows *S. viridis* to adapt to small-scale disturbances that are common in these habitats, but a diet specializing in seagrass may make this animal vulnerable to large-scale seagrass declines occurring worldwide.

Key words: herbivory, gastropod, *Thalassia*, *Halodule*, *Syringodium*

Seagrass beds represent an important habitat for a large number of mollusc species in the world's coastal oceans (Hemminga and Duarte 2000). Recent data show that herbivory on seagrasses is more important than previously thought, yet the role of small seagrass-feeding invertebrates is still poorly understood (Heck and Valentine 2006). Because most seagrass-associated molluscs (*i.e.*, gastropods) and crustaceans (*i.e.*, amphipods and isopods) feed heavily on epiphytic algae attached to seagrass leaves (Jernakoff *et al.* 1996), small epifauna that also obtain nutrition directly from living seagrass may have a competitive advantage over other mesograzers. Predation on these small seagrass-feeding invertebrates may represent an additional pathway in the mobilization of seagrass carbon from plant material to animals in higher trophic levels, such as crabs and fishes (Castriota *et al.* 2005).

The emerald neritid *Smaragdia viridis* (Linnaeus, 1758) inhabits seagrass beds of the Caribbean Sea, Mediterranean Sea, and probably the western African coasts. This large geographic range seems to be explained by the species' planktotrophic development with larvae remaining in the water column up to 55 days (Scheltema 1971). In the temperate meadows of the Mediterranean Sea, this small gastropod resides in *Cymodocea nodosa* and *Zostera marina* meadows, where leaf epidermal tissues of these plants are its main food source (Rueda and Salas 2007). In these meadows, densities may reach 50 individuals m^{-2}, with maximum values in the summer due to enhanced recruitment (Rueda *et al.* 2009). In the subtropical to tropical meadows of the Caribbean, *S. viridis* has been shown to associate with *Thalassia testudinum* (Scheltema 1971, Lewis and Hollingworth 1982, Thayer *et al.* 1984, Sterrer 1986, Brunt and Davies 1994). The emerald neritid is also found in *Halodule wrightii* and *Syringodium filiforme* pastures (K. Holzer, pers. obs.). The association of *S. viridis* with Caribbean seagrass meadows suggests a possible trophic dependence. In a paper published by Thayer *et al.* (1984: 362), the authors recount,"*S. viridis* roams about the lower half of the green [*Thalassia*] blades and removes a furrow about 1 mm wide and half the thickness of the blade with its radula." A comment on the presence of seagrass epidermal tissues in feces of *S. viridis* was also made in a paper characterizing the epifauna of *Thalassia* meadows in Barbados (Lewis and Hollingworth 1982). However, these two studies did not deal with ingestion of tissues of different seagrass species by *S. viridis*. We addressed this issue in a laboratory experiment where we presented *S. viridis* with tissues of three subtropical to tropical western Atlantic seagrass species (*T. testudinum*, *H. wrightii*, and *S. filiforme*). Bermuda represents the northernmost limit of these marine angiosperms. We documented the radular marks made on the seagrass leaves and quantified the presence of seagrass cells in the fecal pellets produced during the feeding trial. We also obtained the relative proportion of lysed to intact seagrass cells in the feces as a proxy for digestibility. This study provides data refining the often-cited paradigm that small gastropods in subtropical to tropical seagrass meadows ingest seagrass only incidentally while feeding primarily on algal epiphytes.

MATERIALS AND METHODS

A microcosm feeding experiment was conducted in October 2008 to establish diet preferences of the neritid gastropod *Smaragdia viridis* which is a common component of the epifauna assemblage in seagrass meadows of the Caribbean and Bermuda. In Bermuda we recorded snail densities of 0-150 individuals m^{-2} in different seagrass areas, as well as the presence of the characteristic hemispherical (*ca.* 1 mm diameter) glossy-yellow spawn masses deposited on epiphyte-free seagrass blades at the base of the shoot. For this experiment, neritids were collected by snorkelers on Bermuda's north shore from three shallow (*ca.* 2 m depth) mixed-species beds dominated by *Thalassia testudinum*, and also including *Halodule wrightii* and *Syringodium filiforme*: Fort Saint Catherine (32°23′N, 64°40′W), Higgs Cut (32°22′N, 64°39′W), and Clearwater (32°21′N, 64°39′W). All study animals were found attached to one of the three seagrass species and were never observed on rock or algal substrates. They were acclimated to laboratory conditions for a week and supplied with abundant seagrass-epiphyte forage from their local meadows.

The weeklong feeding trial was performed in an outdoor flowing seawater system exposed to ambient temperature, light, and weather conditions at the Bermuda Institute of Ocean Sciences. Individual microcosms consisted of neutrally buoyant 120 ml transparent plastic containers that were perforated with 14 × 3-4 mm holes to permit constant water exchange. One *Smaragdia viridis* was placed inside each container along with a single-species seagrass diet ($N = 10$). Food items consisted of healthy (green) undamaged 5-10 cm long segments of both new and old seagrass leaves (*Thalassia testudinum, Halodule wrightii*, or *Syringodium filiforme*) and attached epiphytes. Food was collected from Bailey's Bay (32°20′N, 64°43′W) at *ca.* 2 m depth, where epiphyte loads were 1.6 ± 0.5 mg DW cm^{-2} and epiphyte organic content was 25 ± 2% (mean ± *SD*, $N = 10$). Fresh forage was exchanged daily for five consecutive days. On the sixth day, the animals were held in separate glass bowls containing seawater filtered through a 45-µm membrane, and feces were recovered after 24 hours with a pipette.

At the end of each experiment, seagrass blades were examined for radular marks created by the neritid using a binocular microscope, and those areas on leaves containing feeding trails were fixed in Lugol. Feces egested by *Smaragdia viridis* during the experiment were also collected using a pipette and fixed in Lugol for further microscopic analyses. Empty seagrass cells (without cytoplasm and chloroplasts) and intact seagrass cells (with cytoplasm and chloroplasts) were counted in fecal pellets collected during the experiment using the three seagrass species. Components of the epiphyte community (macroalgae, diatoms, and foraminifers) in the feces were also tallied. The potential assimilation of the three seagrass species was then estimated as the percentage of empty seagrass cells compared to the total number of seagrass cells (empty and intact).

Separate Chi-square analyses for each seagrass diet were used to evaluate whether snails (1) prefer seagrass tissues or epiphytes and (2) lyse seagrass cell walls to access the cellular contents. The independent variable in both cases was cell type and the dependent variable was abundance of that cell type in the fecal pellet. Expected values were based on a predicted equal number of (1) seagrass and epiphyte cells and (2) empty and intact seagrass cells, for each of the three seagrass diets. While statistical tests were run on cell abundance, we report the percentage of the different cell types comprising fecal pellets to convey more clearly significant trends in the data.

Smaragdia viridis makes consistent and characteristic grazing trails, independent of season. The weeklong feeding trial was repeated in July 2009 to obtain seagrass and feces samples to photograph.

RESULTS

Smaragdia viridis left radular marks while eating the epidermal tissues of all three seagrass species (Figs. 1A, 1D, 1G). Radular marks were made along the central part of leaves (up to 5 cm long), except for *Syringodium filiforme*, which has a circular leaf cross-section. In *Thalassia testudinum*, the radular marks were less continuous than in both *S. filiforme* and *Halodule wrightii,* and sometimes epidermal tissues of both sides of the *T. testudinum* blade were ingested producing transparent lines (Fig. 1A).

In all cases, fecal pellets were cylindrical (200–400 µm length) with a longitudinal green line (10–20 µm width) rich in chloroplasts. Under microscopic observation, the feces consisted mainly (> 95%) of tissues of the three seagrass species, which were easily distinguished by cell size and shape (Fig. 1C, 1F, 1I). Ungrazed leaves of the three different seagrass species are shown for comparison (Figs. 1B, 1E, 1H). Epiphytes (macroalgae, diatoms, and foraminifers) represented < 3% of the fecal composition and were significantly lower in abundance than seagrass cells (empty and intact) for all seagrass treatments (*Thalassia testudinum* $\chi^2 = 45.9$, $df = 1$, $N = 10$, $P < 0.05$; *Halodule wrightii* $\chi^2 = 15.1$, $df = 1$, $N = 10$, $P < 0.001$; *Syringodium filiforme* $\chi^2 = 23.0$, $df = 1$, $N = 10$, $P < 0.001$) (Fig. 2A). The abundance of empty seagrass cells was significantly higher than intact cells for *Thalassia testudinum* ($\chi^2 = 18.2$, $df = 1$, $N = 10$, $P < 0.001$) and *Halodule wrightii* ($\chi^2 = 9.4$, $df = 1$, $N = 10$, $P < 0.01$), but not for *Syringodium filiforme* ($\chi^2 = 3.3$, $df = 1$, $N = 10$, $P > 0.05$) (Fig. 2B).

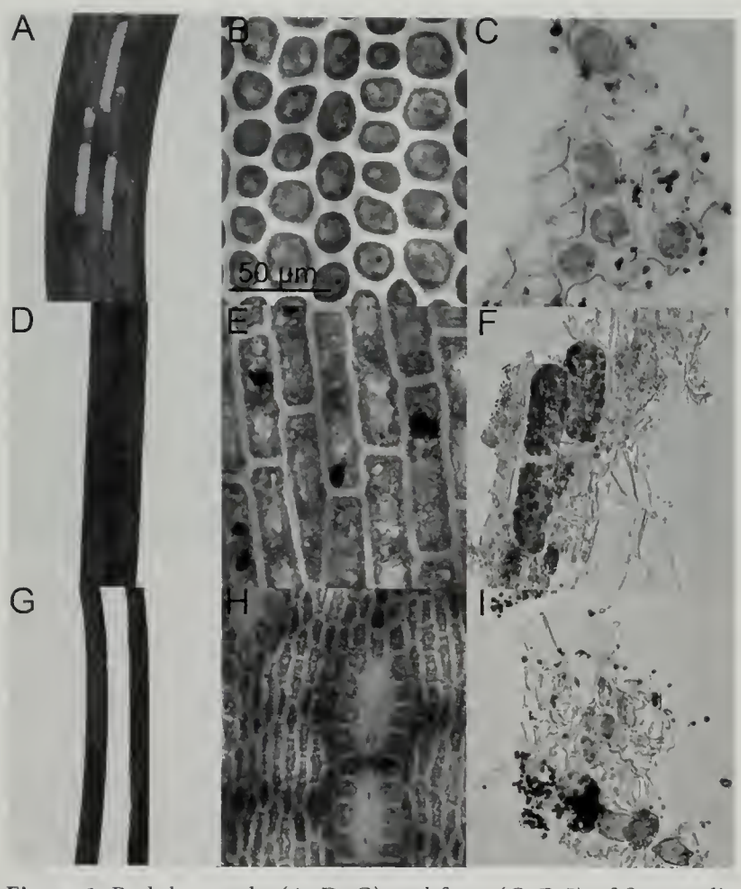

Figure 1. Radular marks (A, D, G) and feces (C, F, I) of *Smaragdia viridis* after grazing *Thalassia testudinum* (A–C), *Halodule wrightii* (D-F), and *Syringodium filiforme* (G-I). Unaltered cells of leaf tissues from *T. testudinum* (B), *H. wrightii* (E), and *S. filiforme* (H) are shown for comparison. Scale bar is the same for feces and unaltered cells of seagrass leaf tissues. *Thalassia testudinum* leaf width: 7.1 mm; *H. wrightii* leaf width: 1.8 mm; *S. filiforme* leaf width: 0.8 and 0.9 mm.

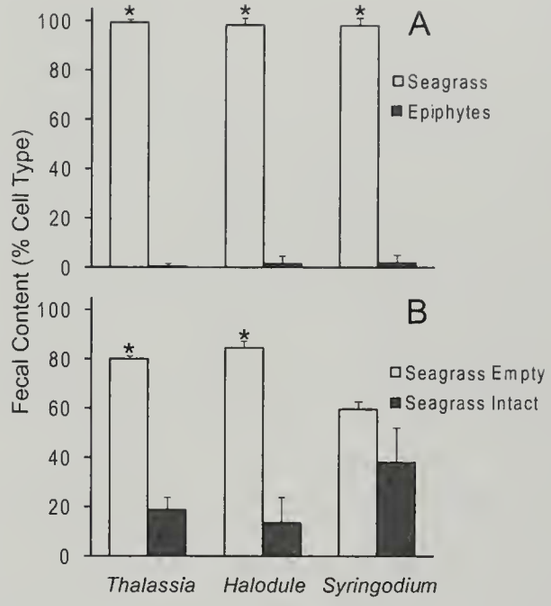

Figure 2. Mean (+ *SD*) fecal content of *Smaragdia viridis* after feeding on three different seagrass species. (A) Percentages of seagrass (empty and intact) and epiphyte (macroalgae, diatoms, and foraminifers) cells in snail feces (N = 10). (B) Percentages of empty (without cytoplasm) and intact (with cytoplasm) seagrass cells in snail feces (N = 10).

DISCUSSION

We present experimental data that *Smaragdia viridis* feeds on the Caribbean seagrass species *Thalassia testudinum,* which confirm that this gastropod directly ingests seagrass tissue and support the brief anecdotal observations in Thayer *et al.* (1984) and Lewis and Hollingworth (1982). Our laboratory data show that this neritid also feeds on at least two other Caribbean seagrasses, including *Halodule wrightii* and *Syringodium filiforme*, Our results support an emerging trend of direct feeding on seagrass by other *Smaragdia* species. Seagrass-dependence has been documented for *S. bryanae* (Pilsbry, 1917) with *H. hawaiiana* in Hawaii by Unabia (1980) and for *S. souverbiana* (Montrouzier, 1863) with *H. ovalis* and *H. spinulosa* in eastern Australia (J. Rueda, pers. obs.). In the Mediterranean Sea, *S. viridis* is considered a different subspecies (*S. viridis viridis* Linnaeus, 1758) than in the Caribbean Sea and it also feeds on seagrasses, but of different genus and species, such as *Zostera marina* and *Cymodocea nodosa* (Rueda and Salas 2007). Other *Smaragdia* species also strictly associate with seagrasses, such as *S. rangiana* (Récluz, 1841) with *Thalassia hemprichii* in the Republic of Seychelles (Taylor and Lewis 1970), but information on feeding is presently unavailable. There are also less common *Smaragdia* species, such as *S. tragena* (Iredale, 1936) or *S. roseopicta* (Thiele, 1930), for which habitat preference is undocumented (Loch 1994), and more work is clearly needed to confirm that all species in the *Smaragdia* genus associate with and feed on seagrasses.

We also demonstrate that *Smaragdia viridis* grazing can rupture plant cell walls, providing access to the more nutritious cell contents. Previous studies in marine and terrestrial ecosystems suggest that grasses coevolved with herbivores by defending themselves through producing high silica and fiber levels in their cell walls, and that some herbivores (*e.g.*, mammals, fishes) have evolved special dentition to accommodate plant toughness (review, Thayer *et al.* 1984). Our data indicate that *S. viridis* uses a specialized feeding technique to break apart plant cell walls and empty the more palatable cell content inside. By using the radula to destroy the physical integrity of the cells, the snails can gain access to the cytoplasm without necessarily having to digest the cellulose of the cell wall or rely on symbiotic gut flora. It is possible that other small invertebrates also use the same process to destroy cell integrity and ingest the cytoplasm, and that this is a mechanism by which substantial energy enters higher trophic levels.

While some of the palatable cytoplasm may leak into the environment during feeding, evidence that *Smaragdia viridis* assimilates living seagrass tissues comes from isotopic data collected in August 2008 showing that emerald neritid tissues contain $\delta\ ^{13}C$ values (-8 to -9 ‰) close to those of green *Thalassia* leaves but significantly more enriched than the epiphyte-periphyton consortium attached to the leaves (K. Holzer, unpubl. data). Seasonal variability in epiphyte loads and epiphyte composition are not expected to alter the contribution of seagrass to the diet of *S. viridis* since field observations indicate that the emerald neritid targets emergent epiphyte-free leaf tissue at the base of shoots (Thayer *et al.* 1984, Rueda *et al.* 2009, K. Holzer, pers. obs.).

Information on ingestion of food is limited in marine grazing gastropods compared to other molluscs (*e.g.*, filter feeding bivalves) (Saleuddin and Wilbur 1983), but absorption of ingested food has been positively correlated with the quality of the food in bivalves (Iglesias *et al.* 1996, Hawkins *et al.* 1998). Our data indicated that the proportion of broken over intact seagrass cells egested was higher only for *Thalassia testudinum* and *Halodule wrightii*-fed snails and not for *Syringodium filiforme* (Fig. 2B). A different pattern was found for *S. viridis* feeding on *Zostera marina* and *Cymodocea nodosa*, with lower percentages of broken cells for the later (J. Rueda, unpubl. data). This relationship may be linked to a higher amount of cell wall material in relation to cell cytoplasm in *C. nodosa* compared to *Z. marina*. Fittingly, the three tropical Caribbean seagrasses contained different cell sizes with the largest cells for *H. wrightii* followed by *T. testudinum* and the smallest cells for *S. filiforme* (Fig. 1B, 1E, 1H). A large number of small cells may result in compact tissue that is more difficult to digest than tissues consisting of much larger cells with a lower combined surface area and lower proportion of lignified cell wall material. Alternatively, leaves of *S. filiforme* are rounded not flattened like the other two seagrasses, which could impede the grazing process.

In summary, *Smaragdia viridis* shows diet flexibility among seagrass species and can consume the pioneer Caribbean seagrasses *Halodule wrightii* and *Syringodium filiforme* as well as the climax species *Thalassia testudinum*. Grazing along the succession sequence of seagrass makes *S. viridis* suited to recovery following small local disturbances. However, this seagrass-dependent snail may also be susceptible to accelerating trends of global seagrass decline (*i.e.*, Waycott *et al.* 2009). Conversely, the formation of lesions on live seagrass blades from the rasping activity of these snails (Fig. 1A, 1D, 1G) may suppress plant growth as has been shown for the periwinkle *Littoraria irrorata* (Say, 1822) on salt marsh grass (Silliman and Zieman 2001) or the limpet *Tectura depicta* (Hinds, 1842) on eelgrass (Zimmerman *et al.* 1996). The radula of *S. viridis* may also puncture lacunal air channels that transport gases from seagrass leaves to roots, causing leakage (Fig. 3) and an interruption of oxygen delivery to the rhizosphere. We suggest that a reduction of predatory crabs or fishes, through overfishing, could increase the abundance of *S. viridis* and exacerbate the potentially harmful impacts of neritid feeding on the seagrass host.

Figure 3. *Smaragdia viridis* actively feeding on a *Halodule wrightii* shoot. Arrows identify bubbles leaking from the new grazing-lesion.

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